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TECHNICAL REPORT NO. 1

A Role for Cytoplasmic Structural Proteins in the
Transport of Water and Salts in the Intestine

by

Paula T. Beall, Ph.D.

Department of Physiology
Baylor College of Medicine
1206 Moursund
Houston, Texas 77030

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A Role for Cytoplasmic Structural Proteins in the
Transport of Water and Salts in the Intestine

February 1, 1981 - January 31, 1984

This contract supports research to test the hypothesis - that the movement of water and salts across the epithelial membrane of the intestine depends not only on the properties of the cellular membranes but also on the structural properties of the cytoplasm of the cells.

Current theories ascribe all controls of cellular transport of water and salts to passive diffusion and specialized enzymes, pores, and channels at the cell membrane. Classical interpretation allows no role for the cytoplasmic components of the cells in regulation or control of transport. Scattered experimental evidence produced in the 1970's, however, suggests that the disruption of the microtubule and microfilament network of the cytoplasm can have an effect on the rate and direction of transport. This proposal is in the process of testing the idea that the cytoskeleton plays a role along with water to regulate transport. For this purpose the intestinal epithelium has been chosen as a membrane through which large amounts of sodium and water pass. This work is part of a coordinated effort among three labs to study this phenomenon in vivo in perfused rat intestine, in vitro on stripped intestinal mucosa in Ussing chambers, and in isolated intestinal enterocytes. Preliminary data produced by this team has already resulted in the acceptance of a role for cytoplasmic proteins in the electrical properties of the intestinal epithelium.

YEAR 1

In Year 1 the proposal is scheduled to address the proper procedures for the isolation of intestinal enterocytes in a viable state for subsequent transport studies in which the effects of cytoskeletal disrupting drugs will be tested.

1. Progress on perfusion of rat intestine in vivo: Before experiments to isolate enterocytes can begin base line parameters for the normal operation of the cells in the intact intestine must be determined. In collaboration with Dr. Cassidy, I have performed a sample of these experiments proving that cytochalasin B which disrupts actin filaments in cells does indeed reduce water and salt transport across the epithelium. I am continuing these experiments.

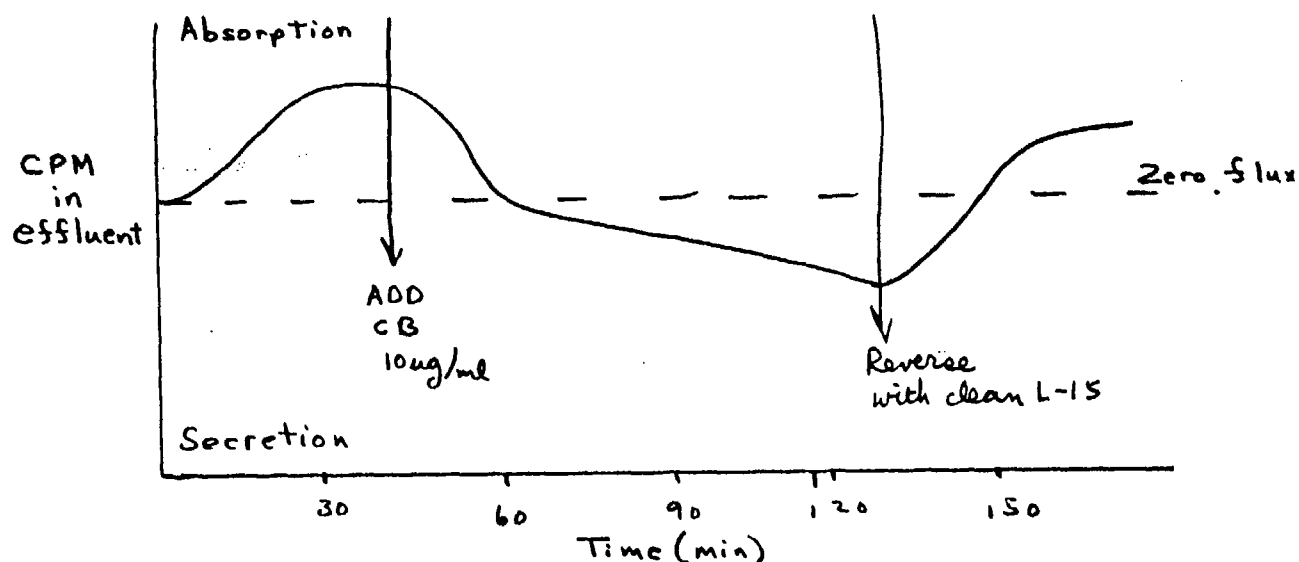
Methodology

- (a) L-15 medium: Because of its ability to sustain isolated epithelial cells in tissue culture and our preliminary experiments on isolation (section 3) we wish to use L-15 culture medium for the cell experiments. We must know how this effects transport in vivo.

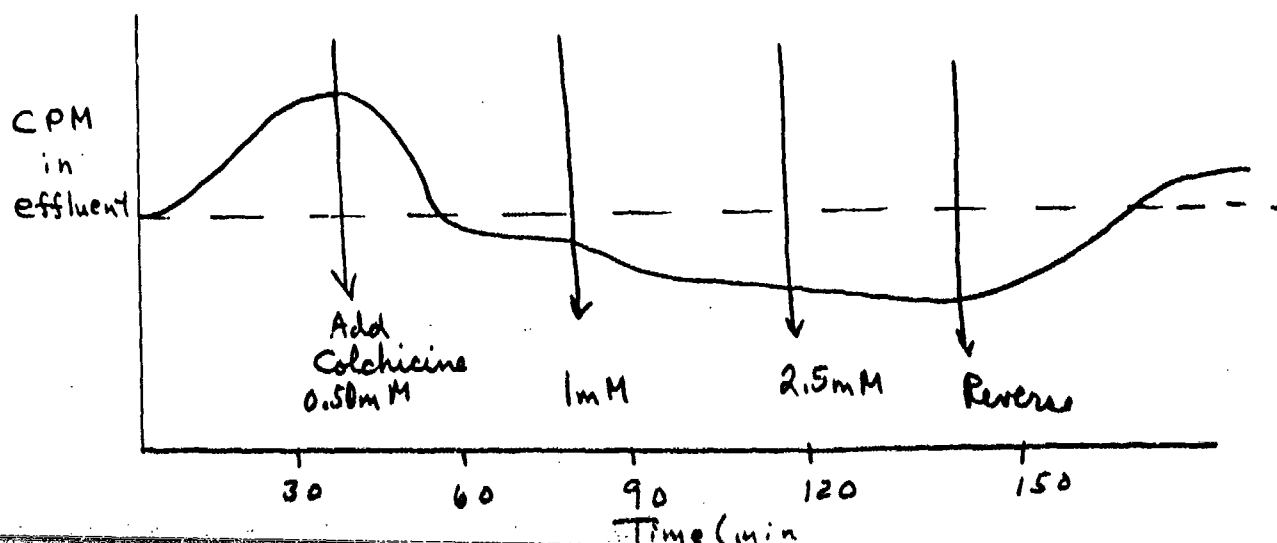
1. It has been determined that the magnitude of water transport in normal intestine is greater with L-15 than with a balanced buffered salt solution.
2. It is possible that the variety of amino acids in L-15 stimulate transport.

Data

- (b) Effects of Cytochalasin B on transport in vivo: Experiments were done in which the intestine is first perfused with L-15 containing ^{14}C -PE6 as a volume marker. Absorption at normal levels was demonstrated. Then L-15, (^{14}C -PE6) was perfused through the intestine containing cytochalasin B at doses of 10 to 100 mg/ml. All doses caused secretion as predicted by the hypothesis:

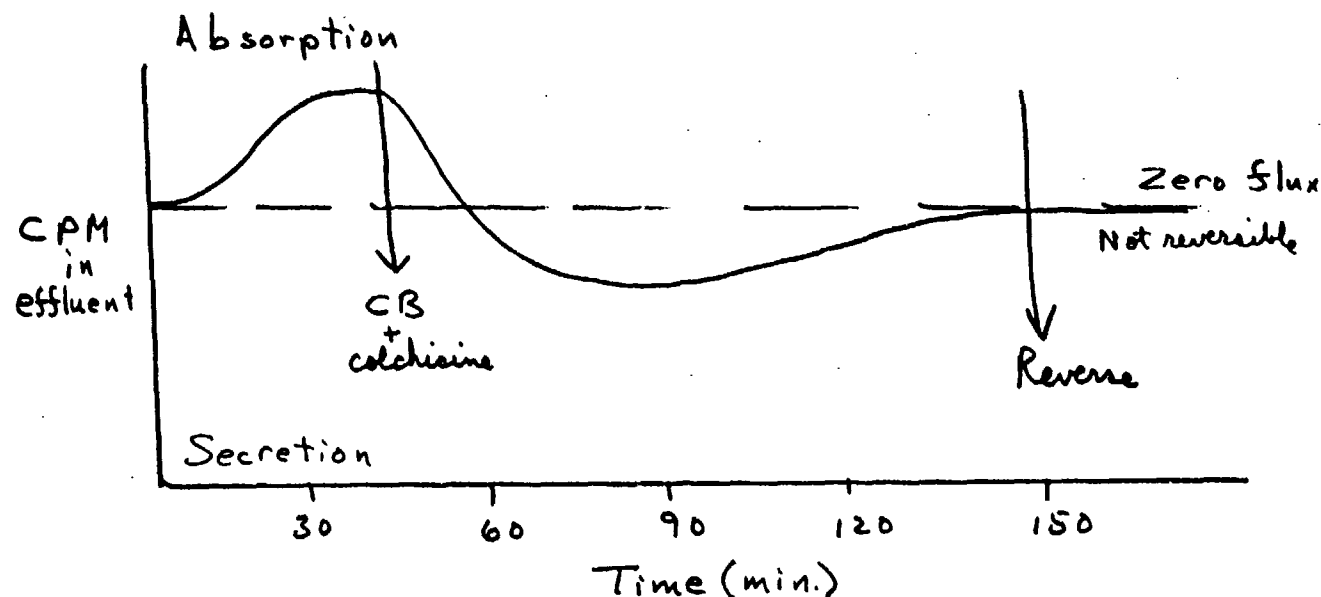


- (c) Effects of Colchicine on transport in vivo: Experiments were done in which the intestine was perfused for control absorption and then with L-15 containing colchicine, a drug which disrupts microtubule polymerization.



A dose dependent response was seen for 0.5-2.5 mM colchicine. Secretion occurred as predicted by the hypothesis but to a lesser extent than with much lower doses of CB. Return to normal L-15 was able to slowly reactivate absorption over 1 hour.

- (d) Combination of CB and colchicine: Experiments were done in which intestine was perfused with L-15 to establish control absorption levels, and then with L-15 containing 20 mg/ml CB and 2.4 mM colchicine. Initially secretion was seen and then after \approx 30 minutes, secretion ceased.



No return to absorption was noted upon perfusion with L-15 containing no drugs. The combined effects of the drugs are not reversible.

Results

- (e) All of the above experiments meet the predictions of the hypothesis that cytoskeletal proteins may play a role in transport in the intestine. These experiments are proceeding and will probably be published in 1982.

2. Progress on the Development of a Method for the Isolation of Viable Rat Enterocytes:

The experiments proposed in this project require large amounts of viable functioning cells from the surface of the small intestine. While methods had been developed for the isolation of enterocytes, these procedures produced cells that showed signs of morphological and biochemical damage. Experiments to determine the proper temperature the best isolation medium, and the oxygen tension requirements of the cells. These results are summarized in Table II.

TABLE II

Viability of Rat Enterocytes

Conditions of Isolation	% Cells Excluding Trypan Blue
L-15 Tissue Culture Medium bubbled with 95% oxygen- 5% CO ₂ (with or without serum)	90%
L-15 plus oxygen (preincubated with trypsin enzyme 1 hour)	80-90%
L-15 plus oxygen (preincubated with 0.05% collagenase for 1 1/2 hours)	70-80%
L-15 <u>NO oxygen</u>	50-70%
Buffer and 0.05% Hyaluronidase	20%
Balanced salt solution and EDTA	10-20%
Ringers Lactate	10-20%

- Conclusions:
1. Balanced salt solutions previously used for the isolation of chick enterocytes are not good for mammalian enterocytes.
 2. L-15 tissue culture medium which was designed to support colon and breast epithelial cells provides additional nutrients needed by these mammalian cells.
 3. The inclusion of oxygen dramatically increases short term survival.

These results were presented at the FASEB spring meetings 1981. In the same session were several people who had been attempting to isolate mamalian enterocytes with little success. All are going to try L-15, all agreed that salt solutions are not sufficient. In addition after these results were published another paper came out in Epithelial Transport of Water and Ions, W. Armstrong ed. 1981, extolling the virtues of L-15 culture medium for transport studies.

4. I will use L-15, plus oxygen at 25°C for my isolations.

3. Examination of Human Colon Cell Lines as Potential Model Systems:

Because a great deal of water and salt are readsorbed in the colon, the cells that line this organ might be of interest in the study also. In collaboration with Dr. L.P. Rutzky, of the University of Texas Medical School, Houston, I have examined the water and ion content and the NMR relaxation times of a number of normal and cancerous human colon cell lines. The results of the study are summarized in Table I.

TABLE I

Cell Line	(n)	Passage No.	Division Time-hrs	T ₁ (msec)	T ₂ (msec)	% Water
NORMAL COLON CELLS						
Adult NBV	(2)	12	24	1214±46	207±7	92.3±0.4
Adult NBM	(3)	13	24	1009±7	191±3	91.4±0.4
Fetal HSO677	(2)	20	20	1058±13	221±3	90.6±0.4
Fetal Hs0074	(2)	27	22	1106±24	163±23
CANCER COLON CELLS						
LS 180	(5)	41	24	643±60	119±12	85.5±2.4
LS174T	(6)	37	20	744±16	121±3	86.6±0.5
Clone 3-5	(6)	93	19	663±65	127±11	89.7±1.9
Clone 6-6	(7)	60	20	716±39	114±10	86.4±1.2
HT 29	(7)	140	12	686±21	108±7
SW 480	(4)	614	39	982±9	176±6	90.1±1.4
SW 1345	(6)	55	51	460±45	83±6	83.6±1.8

Conclusions: The normal cells will not continue to grow after a limited number of cell divisions in culture. This will make it difficult to obtain sufficient numbers of cells to test the hypothesis. However enough cells could be obtained to test the effects of the drugs in a complementary experiment to the freshly isolated small intestine cells. The morphological differences among the cancer cells make it unlikely, that they can be used as a model for transporting cells. However some investigators at UT Medical School are using an isolated epithelial cell from the kidney grown on millipore filters to undertake transport studies. These cultured cells may be a better model system.

Significant Achievement of Year 1

- 1.) The laboratory for the contract was set up in new department at Baylor College of Medicine after return from Washington.
- 2.) Methods for in vivo perfusion and isolation of intestinal enterocytes were developed.
- 3.) Experiments in the in vivo perfusion system showed that cytochalasin B and colchicine as cytoskeletal disrupting agents both caused secretion as predicted by the main hypothesis.
- 4.) Model systems of human colon cells were tested and may be used in experiments.
- 5.) Five major publications were published in Year 1.

Plans for Year 2

- 1.) Experiments on in vivo perfusion with cytochalasin and colchicine will be completed and published as background for conclusions on isolated enterocytes. All predictions of the hypothesis have held up so far, so some additional experiments may be conducted on other cytoskeletal disrupting agents.
- 2.) The in vivo model may be used to test drugs which will prevent secretion and restore normal function in secreting intestine. One such drug is "taxol" a newly discovered agent that causes rapid driving of polymerization of microtubule proteins.
- 3.) Sodium and water fluxes in isolated enterocytes will be measured and conditions for short term maintenance determined.
- 4.) Sodium and water fluxes in isolated enterocytes will be determined in the presence of cytochalasin B and colchicine.

CURRENT REPORTS AND PUBLICATIONS

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3. P.T. Beall, S.P. Bowen, M.M. Cassidy and M. Dinno, "Effect of Structural Perturbation by Cytochalasin B on Ion and Water Transport in Tight and Leaky Epithelia. An Electrical Model". J. Physiology (London) 308: 90-91 (1980).
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6. P.T. Beall, "NMR Relaxation Times of Water Protons in Cultured Cells During Freezing and Under Osmotic Stress". In Biophysics of Water, Felix Frank, Editor, Elsevier Holland, (1982).

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1. P.T. Beall, B.B. Asch, D. Medina and C.F. Hazlewood, "Distinction of Normal, Preneoplastic, and Neoplastic Mouse Mammary Cells and Tissues by Nuclear Magnetic Resonance Techniques". In The Transformed Cell, Ivan Cameron and Thomas Pool, Editors, Academic Press, N.Y. pp. 293-325, (1981).
2. P.T. Beall, D. Medina and C.F. Hazlewood, "The Systemic Effect of Elevated Tissue and Serum Relaxation Times for Water in Animals and Humans with Cancers". In NMR Basic Principles and Progress, P. Diehl, E. Fluck and R. Kosfeld, Editors, Springer-Verlag, Berlin, Vol. 19, pp. 38-57, (1981).

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2. P.T. Beall, L.K. Misra, C.F. Hazlewood and R.L. Young, "Hormone Dependent Changes in NMR Relaxation Times in Tissues of Aged Female Rats". Biophysical Society, (1982).
3. P.T. Beall, "Isolation of Rat Intestinal Enterocytes in L-15 Medium". Fed. Proc. 40(3): 420a, (1981).
4. P.T. Beall, D.C. Chang and C.F. Hazlewood, "Proton NMR Relaxation Times of Water in Contact and Non Contact Inhibited Cells". Biophysical Journal (1981).
5. M.A. Dinno, S.P. Bowen, P.T. Beall and M.M. Cassidy, "Effects of Cytochalasin B on the Properties of Frog Gastric Mucosa". FASEB (1980).

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In Year 1, of this contract the following significant findings have been made: 1) cytochalasin B, an actin depolymerizing drug, causes the onset of a secretory state in rat intestine at doses from 10-100 mg/ml, 2) colchicine, a microtubule disrupting drug, caused secretion at doses of 0.5-2.5 mM, but to a lesser extent than CB, 3) effects of CB and colchicine are reversible, 4) combinations of CB and colchicine initiate secretion but then stop all secretion and absorption within 1 hr. These findings are consistent with the hypothesis that cytoskeletal proteins play a role in transport.

In Year 2, experiments are planned to test the hypothesis in isolated intestinal enterocytes.

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